CHAPTER 1
Introduction

Contents

1.1. Introduction

Citrus fruit juices hold a significant position as one of the primary juice products in food industries. It exhibits anti-inflammatory, antioxidant, antiviral and antifungal activities due to having nutritive phytochemicals such as octanol, hespiridin, neohespiridin, nomilin, limonin, etc [1]. Several life–threatening diseases such as cancer, cardiovascular disease, heart stroke and reduction in blood glucose have been prevented with the influence of citrus species [2, 3]. Fruit peel, a waste product to the juice industry, is also a major source of flavonoids, pectin, and essential oils [4]. Most of the citrus fruits such as kinnow (a hybrid of two citrus cultivars — 'King' (Citrus nobilis) × 'Willow Leaf' (Citrus × deliciosa)), mosambi (Citrus limetta), orange (Citrus sinensis), pomelo (Citrus maxima or Citrus grandis), lemon (Citrus limon), galgal (Citrus pseudolimon), tangerine (Citrus reticulata) and grapefruit (Citrus × paradisi) grown by Indian farmers belong to the Rutaceae family. Due to their nutritional and medicinal values, it has gained huge interest as an essential food item. But there are significant issues with "bitterness" while processing the citrus fruit juices, as a result of which consumers' acceptability of juice products decreases. The decreased market value of bitter juices creates significance in the economy also. There are two different ways by which bitterness occurs in citrus fruits, a) the flavonoids and their derivatives that causes bitterness (orange, etc) b) bitterness due to the transformation of non-bitter components to bitter ones (mosambi, etc) [5]. Bitterness in citrus fruit juice is caused primarily by two biomolecules, naringin and limonin. The instant bitterness at the moment of juice preparation is due to the naringin of the flavonoid phenolic group whereas bitterness generated during its storage after juice preparation called delayed bitterness is due to the white crystalline biomolecule limonin of the limonoid terpine group [6]. The other bitter phytochemical like nomilin do not significantly contribute to the bitterness problem as its concentration is very much less than that of limonin. Therefore, the detection of limonin is very crucial for effective debittering intrusions. There are many methods available for the quantification as well as debittering of naringin [7] [8]. However, for the detection and debittering of limonin, few works have been reported [9] [10].

Throughout the world, citrus fruits are cultivated extensively but consumer dislike the flavor of the fruit due to the instant bitterness which is primarily for the presence of of naringin in high levels. Besides naringin significant levels of limonin is also present that causes the same problem [11-13]. Around 600 ppm of naringin was found in the fruit during early season, which declines to 100 ppm in the late season in several categories of fruits. To be acceptable to consumers, the detection threshold for naringin should be in the range of 20 to 50 ppm [14]. The phytochemical limonin, a highly oxygenated triterpene derivative causes delayed bitterness in citrus fruits comprised of a furan ring and an epoxide group [15]. Most of the delayed bitterness in grapefruit juice, citrus limetta (sweet lime) and Kinnow mandarin juice is due to limonin. [16, 17]. It is observed that early to mid-season fruits with short maturation time can have limonin levels over 30 ppm which is well beyond the threshold limit of ~ 6 ppm.

The intact fruit contains its precursor, limonate- A-ring lactone (LARL), endogenously in the cell cytoplasm of membranous sacs. The LARL has a pH that is neutral and or slightly alkaline. The LARL encounters the juice's net acidic pH as soon as the sacs are ruptured during juice processing, which initiates the closure of the ring and causes the bitter molecule limonin to be formed through the processes of deglycosylation and cyclization [18-20]. The Limonin D-ring Lactone Hydrolase enzyme present in fruit's albedo accelerates this natural process. The cultivars that are prone to slow bitterness create problem due to this natural process of bittering [21]. The glucosidase enzymes catalyze the transformation of bitter limonin to less or non-bitter end products, thus rendering the citrus juice consumer palatable. Juice acceptance by consumers is influenced by the amount of limonin that is present in the juice. Juice becomes unacceptable when the limonin concentration exceeds the threshold of ~6 ppm [22]. Therefore, it is very important to quantify and detect limonin for improvement in fruit quality and economic growth of the same. By using biochemical and physical means such as radioimmunoassay (RIA), thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and enzyme immunoassay (EIA) for bitterness assessment the limonin can be detect and controlled to a semi-acceptable limit. The rapid and onsite detection of limonin concentration is essential to make the fruit juice product acceptable to the commercial market. Here in our work, the focus has been given to the rapid, accurate and onsite measurement of limonin concentration in citrus fruit juice products.

Here, the design and development of a capacitive sensor based on Interdigitated Electrodes (IDE) structure has been performed to apply the purpose for both detections as well as the measurement of limonin's reduction in citrus fruit juices. Capacitive sensors have been widely used because of their thermal stability (less temperature dependent), simple structure, less power consumption, low cost, etc [23]. The other advantage of using a capacitive sensor over a resistive sensor is the flicker noise associated with the resistive type which may arise due to the charge transfer function between the sensing layer and the sensing electrodes [24, 25]. The capacitive sensor works on the change in dielectric property of its sensing layer which does not involve the transportation of charges. The Interdigital electrode structure used in the work as the capacitive sensor is among the most frequently used periodic electrode structures. The planar interdigitated structure has been used in the work as a capacitive sensor which exhibits a large number of applications due to its quick response, miniature size, less fabrication cost and having no harmful radiation [26]. The geometry of the sensors affects the sensor's sensitivity and hence sensitivity can be improved by optimizing the geometry of the sensor. Hence by adjusting the sensor's surface area, the number of fingers, their spacing, and the spatial wavelength, (which is the distance between the centerlines of adjacent electrodes) the strength of the output signal can be controlled. The dielectric variation of the sensing layer of the IDE device on exposure to target analytes can be employed as an alternative to sensing in food quality monitoring applications [27].

In this work, novel interdigitated electrodes (IDE) based capacitive sensors for the detection and measurement of limonin reduction in citrus fruit juices have been reported. Citrus fruit juices considered for the analysis includes mosambi (Citrus limetta) and pomelo (Citrus grandis) fruit juice samples for their wide availability and great demand as heath nutritive juice. Further, the limonin developed in the mentioned fruit juices makes it difficult for the fruit industries in their long-term storage. The sensor has been developed using CeO_2 nanoparticles (NPs) for the detection of limonin in citrus fruit juices. The CeO_2 NPs were synthesized through a novel route utilizing Dillenia indica (D. indica) aqueous extract by green-synthesis method. The green synthesis method overcomes various limitations present in physico-chemical methods such as the requirement of costly and toxic materials, high temperature and pressure requirement, less biocompatibility and being non-environment friendly [28]. The nanostructure (CeO_2) here enhances the surface area of the sensor leading to an increase in sensitivity. Further, we have also reported an IDE-based capacitive sensor based on magnesium silicate for both detection as well as reduction of bitterness through selective adsorption by the sensor material. No work has been reported so far on both quantification and limonin reduction in citrus fruit juices using same device.

1.2 Motivation

Limonin in citrus juices has been detected and quantified using several techniques and procedures. The majority of the approaches are analytical, including radioimmunoassay (RIA), thin-layer chromatography (TL C), gas-liquid chromatography (GLC), and enzyme immunoassay (EIA) for limonin assessment. Although the analytical techniques have several advantages in the analysis of various compounds, all of the methods have added drawbacks as they alter the chemical composition of the juice, which affects its quality in terms of flavour, texture, and stability. Moreover, these methods are time-consuming, costly, and unsuited for on-site testing. The High Performance Liquid Chromatography (HPLC) analysis for limonin detection uses toxic materials as mobile phage solvents and the analysis procedure is lengthy. The sensors developed to date such as amperometric biosensor [23], organic Electro Chemical Transistor (OECT) based sensor [27] are also expensive, and time-consuming in terms of fabrication. Further, no methods/techniques/device has been reported to date which can be used for the detection of limonin and reduction measurement of limonin through the same device. Therefore, there is a huge scope of research on the development of devices to overcome the present limitations. By observing the advantages present in capacitive sensors based on IDE, this work is motivated to design and develop a capacitive sensor based on Interdigitated Electrodes (IDE) structure for on-site detection of limonin content and its reduction in citrus juices with high accuracy, selectivity and sensitivity.

1.3 Research objectives

The proposed research work aims to design and develop a capacitive sensor based on IDE for the detection and measurement of the reduction of the phytochemical “limonin” which will be achieved by the following objectives,

- Design and simulation of the capacitive sensor based on Interdigitated Electrodes.
- Synthesis, preparation of the sensing materials, its characterization.
- Fabrication of the sensor and implementation of the same in detection and reduction in limonin content in the citrus fruit juices.

1.4 Thesis Organization

In this dissertation, a highly sensitive, IDE-based capacitive sensor using Cerium oxide nanoparticles and a capacitive sensor using magnesium silicate were proposed and fabricated. The sensor's geometry in ease of its fabrication was designed and optimized using software tools. We have synthesized and investigated the chemical characterization of materials and fabricated the sensor. The sensor's performance was analyzed in terms of its accuracy, sensitivity, reusability, selectivity, cost, etc. Finally, the sensors were validated with analytical techniques such as HPLC.

The dissertation is organized as follows:

Chapter 1 introduces this dissertation, mentioning the motivation, objectives, scope of the research and finally thesis organization.

Chapter 2 outlines a detailed description of the literature on citrus fruits, bitterness in citrus fruit juices, and conventional methods, techniques used for detecting the bittering components and its debittering techniques. This chapter overviews the sensors and their classification, with emphasis on the Interdigitated Electrodes based capacitive sensors. This chapter concludes that biomolecule limonin responsible for delayed bitterness can be used as an indicator for delayed bitterness assessment in citrus fruit juices. Various conventional methods and techniques used to quantify limonin are destructive to its test sample, time-consuming, require complex sample preparation with data analysis, expensive, laborious and require a skilled person to operate. It was revealed from the literature survey that the IDEs based capacitive sensor has certain benefits in comparison to other types of sensors such as low cost, simple fabrication steps, larger exposer area, short response time, etc.

Chapter 3 presents an IDE capacitive sensor with its working, mathematical model based on comb fingers structure and simulation for its design parameters such as the metallization ratio, width of the electrodes, gap between the electrode fingers, overlapping length, finger numbers to find out the optimized design of the sensor. A construction design of the capacitive sensor incorporating the role of the traverse and fringe capacitances of the device has been shown to enhance the capacitive response of the sensor due to variation in the dielectric property of sensing film on exposure to analytes. The capacitance of the sensor was observed theoretically corresponding to the change in permittivity of the sensing layers for different values of width and gap of the electrode fingers. Device simulations based on the derived capacitance model provides an effective design for the development of capacitive sensor in sensing applications. We have fabricated the low-cost IDE sensor using the above design values of the design parameters.

Chapter 4 covers a detailed description of the material synthesis/ preparation, material characterization, sensor fabrication and measuring set-up. The CeO_2 NPs with nano-polycrystalline face cubic lattice structure were successfully synthesized using the aqueous extract of Dillenia Indica petals which is used as a sensing material for limonin detection. For the quantification as well as reduction measurement of limonin, another material Magnesium silicate has been introduced and coated over the IDE structure. This chapter describes the embedded circuit for assessment of the target analyte (limonin in citrus fruit juice) using the sensing setup incorporated with the fabricated capacitive sensors, a microcontroller with other circuitry, LCD and PC.

Chapter 5 presents the quantification of limonin in citrus fruit juices using CeO_2 NPs based sensor. The sensing mechanism of CeO_2 NPs is mentioned here. The sensor is calibrated with different concentrations of limonin and based on the calibration technique, the bitterness in citrus fruits has been assessed in terms of limonin content. The sensor performance characteristics in terms of accuracy, selectivity, reusability, sensitivity, response time are described. The HPLC analysis was performed with the real citrus fruit juice samples to find out the accuracy of the sensor. The IDE capacitive sensor based on CeO_2 NPs shows an excellent specificity of measurement for limonin in citrus limetta and citrus grandis fruit samples with a sensitivity of ~ 9.62 ±0.095 μF/ppm, a very short response time of ~ 13 s and reusability with less than 13.8 % degradation of initial capacitance value after one cycle.

Chapter 6 presents a method for the quantification as well as the limonin reduction in citrus juices. By selectively adsorbing limonin from citrus juice samples, a novel capacitive sensor made of magnesium silicate-poly vinyl alcohol ($MgSiO_3.xH_2O$-PVA) composite has been developed for limonin quantification and reduction. The sensor was calibrated with different concentrations of limonin and based on the calibration technique, the sensor with the measuring setup system was used to assess the bitterness of citrus fruit juices at room temperature. The sensor's results were validated with HPLC analysis. Total Phenolic Content, Total Flavonoid Content, Anti-oxidant activity and sensory evaluation for the debittered juice were presented. Finally, the sensor enables both quantifications as well as the measurement of limonin reduction in citrus fruit juices and compared to other techniques, it is distinctive while exposed to the sensing material. The comparison of developed sensors with already existing techniques was presented.

Chapter 7: summarizes our work and contributions and discusses future work for further research.

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